

Study of Creatinine Transport through Chitosan/Pectin/Poly(Vinyl Alcohol) Blend Membranes

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Abstract

Creatinine was the final product of creatine metabolism in skeletal muscle. Increasing of creatinine showed the decreasing of kidney function. Kidney function decreased could be treated by hemodialysis (HD). One of the natural polymer was cellulose which often used as hemodialysis membrane. Chitosan as natural membrane was used as membrane in this research because the structure was almost similar to cellulose. Chitosan was complexed by pectin and poly(vinyl alcohol) (PVA) to fixed mechanical characteristic of a membrane. The objective of this research was to synthesize, characterize, and know efficiency of creatinine transport using chitosan/pectin/PVA blend membranes. The functional groups of synthesized membrane were characterized by FTIR spectrophotometer. The efficiency of optimum creatinine transport was observed by using membrane with chitosan and pectin ratio 70:30 while PVA used 0.5%; 1.0% and 1.5%. The source and acceptor phase resulted were complexed by picric acid and analyzed by *Ultraviolet-Visible* (UV-Vis) Spectrophotometer. The result of membrane synthesized which was analyzed by FTIR Spectrophotometer showed that there is a band broadening on wave number 3448.72 cm^{-1} . It is indicated that there are an overlapped stretching of hydrogen bond -OH on PVA and NH₂ on chitosan. The optimum creatinine 70 mg/L transported using a membrane with 1.5% PVA addition is 59%.

Keywords: Chitosan, creatinine, membrane, pectin, poly(vinyl alcohol)

Introduction

Creatinine is produced in the muscles by the non-enzymatic changes of creatine and phosphor creatinine. The liver has a momentous role in the assembly of creatinine through methylation of guanidine aminoacetic acid. The normal serum creatinine level is 0.5 to 1.0 mg/dL according to diurnal and menstrual variations, pursuit, and diet. Creatinine is used to assess renal function, however, serum creatinine level will not increase until renal function has decreased by at least 50% (Hamilton *et al.*, 1972). In chronic renal failure there is a steady and continued decrease in renal clearance or glomerular filtration rate (GFR), which leads

to the gathering of urea, creatinine and other chemicals in the blood (Levey *et al.*, 2005).

Hemodialysis is one of the renal replacement therapy. In this technique, body waste product like urea, creatinine and free water are removed from the blood, when the kidneys are impaired. The principle of hemodialysis is the diffusion of solutes through a semi permeable membrane (Abraham *et al.*, 2012). Early dialysis membranes were based on cellulose, with cuprophane (a copper-substituted cellulose) being one of the most commonly used early membranes. These were cheap to produce and had advantages of being thin-walled. However, they had the disadvantage of being immunoreactive. They incited an inflammatory response in the patients with activation of complement, neutrophil superoxide production and cytokine induction (such as interleukin (IL)-1) and tumor necrosis factor. This contributed to dialysis symptoms and intolerance, and in the long term may have contributed to dialysis-

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related cachexia. They were also only available in low-flux form. They only allowed the passage of small molecules with very few molecules above a molecularweight (MW) of 5000 gaining passage through the membrane (Kerr and Huang, 2010).

Reported by Ayuni (2013) that the weakness of cellulose membrane characteristic was solved by using chitosan-pectin PEC membrane which yielded transport efficiency 25.24%. Chitosan is a second abundant natural polysaccharide after cellulose. Chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose] is a collective name for a group of partially and fully deacetylated chitin compounds. Due to its biological characteristics unique, including biodegradability, non-toxicity and antimicrobial. Pectin is an edible and water-soluble polysaccharide which consists primarily of d-galacturonic acid with a part of the carboxyl groups being methoxylated. It is widely used in food industry, and its potential for biomedical applications has been also investigated. Ionic interactions occur between polyanions and polycations, leading to the formation of a polyelectrolyte complex (PEC). The other types of interactions that can form between amino groups (chitosan) and carboxyl groups (pectin), such as hydrogen bonds and covalent bonds formed using specific conjugating chemicals (Richert *et al.*, 2004). There is only ionic interaction in chitosan-pectin PEC membrane so that the membrane is not selective to creatinine. This weakness can be solved by synthetic polymer addition like PVA. PVA is a water soluble synthetic polymer. Due to the characteristics of easy preparation, good biodegradability, excellent chemical resistance, and good mechanical properties, PVA has been used in many biomaterial applications (Park and Ruckenstein, 2001). Chitosan contains hydroxyl and amine groups, which is potentially miscible with PVA due to the formation of hydrogen bonds (Miya *et al.*, 1983). The crosslinking reaction of chitosan with pectin is done in order to incorporate the reactive -COOH groups of pectin to the backbone chain of chitosan. The substitution is expected to increase the active sites on the

surface of chitosan membrane, through creatinine can be transported via hydrogen bonds formation. The mechanical properties of the membrane can be improved by decreasing the chitosan cross linked with pectin hydrophilicity. It can be achieved by mixing the composite with the PVA. The appropriate ratio of the hydrophilic groups and the hydrophobic groups is expected to produce a membrane with high mechanical strength and flexibility (Lusiana *et al.*, 2016).

The objective of this research was to synthesize, characterize, and measure the efficiency of creatinine transport using chitosan/pectin/PVA membrane. The functional groups of synthesized a membrane were characterized by FTIR Spectrophotometer. The efficiency of optimum creatinine transport was observed by using membrane with chitosan and pectin ratio 70:30 while PVA addition 0.5%; 1.0% and 1.5%. The source and acceptor phase resulted were complexed with picric acid and analyzed by UV-Visible Spectrophotometer.

Materials and Methods

Materials

Chitosan, poly (vinyl alcohol), pectin, aquadest, 0.4 M acetic acid solution (CH_3COOH), 0.4 M sodium hydroxyde (NaOH) solution, 8.8 mM picric acid, 0.1 M hydrochloric acid, phosphate buffer solution pH 7.4. All chemicals were analytical grade and purchased from P.T. Merck.

Methods

Synthesis of chitosan/pectin/PVA blend membranes

Ayuni (2013) has synthesized chitosan-pectin PEC membrane that applied in creatinine transport. The maximum creatinine transport with chitosan-pectin composition (70:30) was 25.24%. To increase membrane selectivity in this research will synthesize chitosan/pectin/PVA membrane with chitosan-pectin composition (70:30) was used with a variation of PVA addition 0.5%; 1%; and 1.5%. For the chitosan/pectin/PVA blend membranes synthesis, 0.03 g pectin was

Table 1. Composition of chitosan, pectin, and PVA.

Membrane Type	Chitosan (g)	CH ₃ COOH (mL)	Pectin (g)	Aquadest (mL)	PVA (g)
M1	0.07	16	0.03	4	0.1
M2	0.07	16	0.03	4	0.2
M3	0.07	16	0.03	4	0.3

dissolved in 4 mL aquadest, and then pectin solution was added to 0.07 g chitosan and PVA solution (Table 1). This mixture was stirred for 24 h at room temperature. About 10 mL of the solution was put into petri dish and dried in the oven at temperature 70°C. Afterwards, 0.4 M NaOH was added to remove the membrane from petri dish. The membrane was washed by distilled water until neutral and then dried in open air.

Characterizations of chitosan/pectin/PVA blend membranes

The properties of the membrane were determined by analyzing the material (chitosan, pectin, PVA) before and after blending. Functional groups were obtained by using FTIR Spectrophotometer (Shimadzu).

Water uptake test

To measure the water uptake capability of the porous membranes, the wet weight (W_{wet}) of a membrane was measured, and then the wet membrane was dried in an oven at 60°C for 24 h, followed by vacuum drying for 24 h. The dry weight (W_{dry}) of the membrane was immediately measured. The water uptake was calculated using Eq. 1.

$$\text{Water uptake (\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100 \% \quad \text{Eq. 1}$$

Study of creatinine transport with chitosan/pectin/PVA blend membranes

Transport test was performed using a transport device with the membrane sited in the middle of device. Transport process experiment was done by using the following creatinine concentration 70, 100, and 130 mg/L. The source phase contained 50 mL creatinine standard solution with phosphate buffer addition, while the acceptor phase only contained 50 mL aquadest and phosphate

buffer. The transport tests were carried out for 6 h with samples from source and acceptor phase taken out every 2 h for analysis using UV-Vis spectrophotometer. For the purpose of analysis, creatinine was initially complexed with picric acid. This complex solution was measured at 516 nm. Each membrane and concentration variation was performed twice repetition. The transport efficiency creatinine (E) was calculated using Eq. 2.

$$E (\%) = \frac{\text{Concentration}_{\text{acceptor}}}{\text{Concentration}_{\text{source}}} \times 100 \% \quad \text{Eq. 2}$$

Results and Discussion

Synthesis and characterization of chitosan/pectin/PVA blend membranes

The membranes were synthesized using phase inversion method, in which the liquid phase is converted to a solid phase by evaporating the solvent by gradual heating at a temperature range of 40–70°C for 24–48 h (Lusiana *et al.*, 2016). Membranes was characterized of its functional groups by FTIR spectrophotometer and water uptake.

FTIR spectra analysis

The membrane functional groups were characterized by using FTIR spectrophotometer (Figure 1). In the IR spectra

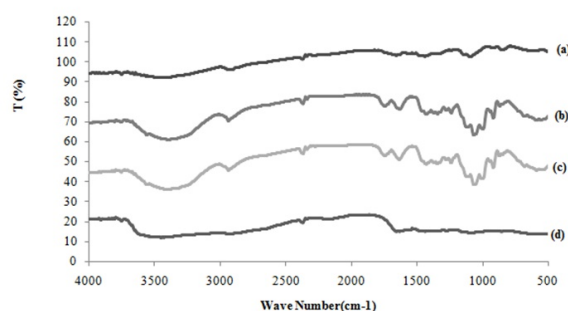


Figure 1. FTIR spectra: a) PVA, b) chitosan, c) pectin, d) membranes.

chitosan, stretching vibration of the amide group of chitosan appears at 1604.77 cm^{-1} . The change in the characteristic shape of the chitosan spectrum as well as shifting of peak to a lower frequency range due to hydrogen bonding between $-\text{OH}$ of PVA and $-\text{OH}$ or $-\text{NH}_2$ of chitosan is evident in membrane. In the spectrum of membrane two relatively broadbands appear in the region $2000\text{--}1600\text{ cm}^{-1}$ which can be ascribed to the overlapping of bands due to amino and carboxylic groups of the interacting molecules. A strong peak at 1635.64 cm^{-1} (asymmetric stretching vibration of carboxylate) also indicates the interchain or intermolecular ionic salt bonds, i.e. polyelectrolyte complex (PEC) between amino groups of chitosan and carboxyl groups of pectin (Rashidova *et al.*, 2004). In the other words, it has been assumed that PEC formation proceeded at the expense of electrostatic interaction between the positively charged amino groups on C2 of the chitosan pyranose ring and the negatively charged carboxyl groupson the C5 of the pectin pyranose ring. Absorption at 1273.30 and 1327.07 cm^{-1} wave numbers only appear on the membrane spectrum. It is thought to be derived from an ester group showing alginate and PVA crosslinked (Lusiana *et al.*, 2013). From the FTIR results, the hypothetical structure of the membrane is presented in Figure 2.

Water uptake

For materials contacting human blood, the balance between hydrophilic and hydrophobic was important (Lusiana *et al.*, 2016). Water absorption of the membrane depends on the structure and composition of the polymer membrane (Huangfu *et al.*, 2009). In this study, membranes were made

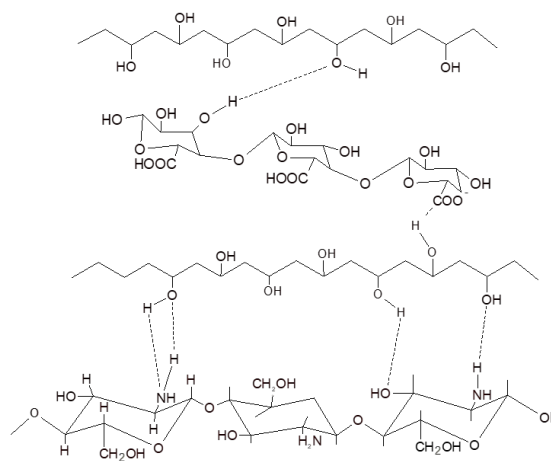


Figure 2. Hypothetical structure of the membrane.

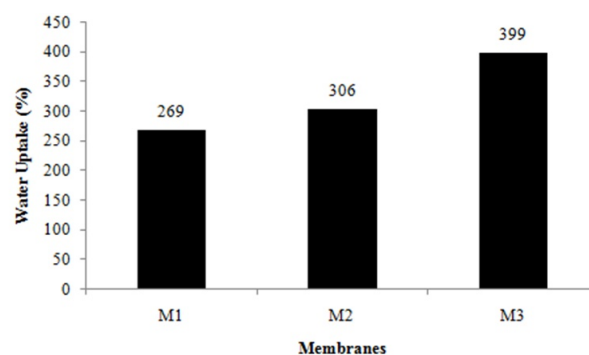


Figure 3. Water uptake of membranes.

of three different PVA addition 0.5% (M1), 1% (M2), and 1.5% (M3). The water uptake (Table 2 and Figure 3) indicated that membranes with 1.5% PVA have the highest water absorption capacity, which amount to 399%.

This value relates to the ability of water diffusion to all parts of the membrane. The appropriate ratio of the hydrophilic groups of chitosan and the hydrophobic groups of PVA is expected to produce a membrane with high mechanical strength and flexibility (Lusiana *et al.*, 2016). This is due to the fact that when a biopolymer contacts with the liquid, there is

Table 2. Water uptake percentage with different composition membrane.

Membrane	Replication			WU (%)
	I	II	III	
M1	269	271	268	269 ± 1.50
M2	306	305	307	306 ± 1.04
M3	399	400	399	399 ± 0.52

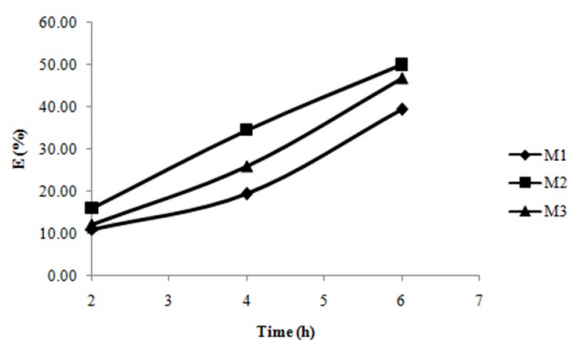


Figure 4. Transport efficiency creatinine 130 mg/L.

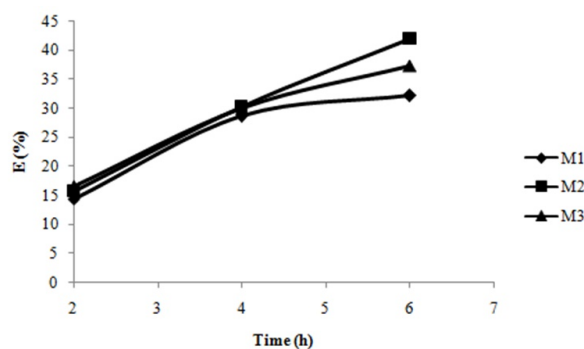


Figure 5. Transport efficiency creatinine 100 mg/L.

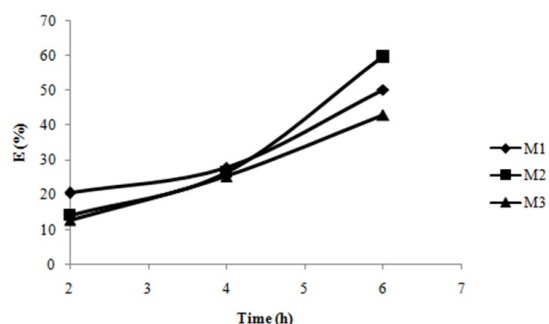


Figure 6. Transport efficiency creatinine 70 mg/L.

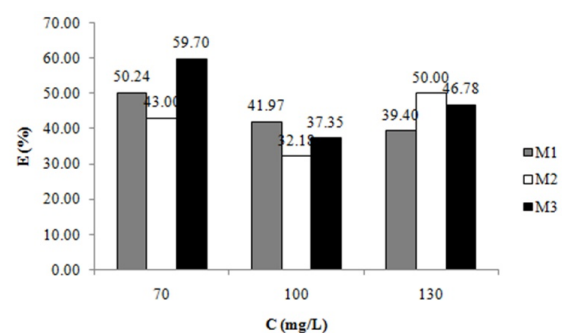


Figure 7. Creatinine efficiency transport for membranes with different composition at transport time 6 h.

a development due to the corresponding thermodynamics and the presence of tensile forces caused by the cross effect that occurs in the polymer chain. As the membrane expands, the mobility of the polymer chain increases to facilitate the penetration of the solvent, in addition to the small ions trapped within the diffused membrane leaving the membrane thus providing a greater opportunity for the solvent to fill in the abandoned empty spaces. The development of the chitosan-alginate membrane is probably due to the ionic interaction between the NH_3^+ group of chitosan and the -COO^- ions of the membrane alginate (Dash *et al.*, 2011; Sagnella and Mai-Ngam, 2005). The blend membranes usually exhibited higher water absorption degree than the

chitosan-pectin membrane (255%) (Ayuni, 2013), indicating a more flexible membrane structure (Huangfu *et al.*, 2009; Gao *et al.*, 2014).

Study of creatinine transport with chitosan/pectin/PVA blend membranes

From Figure 4-6 and Table 3, it can be seen that the longer the transport time, the higher the transport percentage. Furthermore, the transport efficiency percentage tends to increase with increasing amount of PVA in the membrane. It can be seen from Figure 3, that the greater content PVA in blended membranes will increase the ability water uptake of membrane.

In other words, increasing PVA content in the membrane so that increasing

Table 3. Creatinine efficiency transport for membranes at transport time 6 h.

Membrane	E (%)		
	70 (mg/L)	100 (mg/L)	130 (mg/L)
M1	50.24±0.00	41.97±0.00	39.40±0.28
M2	43.00±0.28	32.18±1.27	50.00±0.71
M3	59.70±0.57	37.35±0.00	46.78±0.57

the hydrophilicity of the membrane. It is also correlated with an increase in creatinine transport capability. This might be due to the fact that the addition of PVA into chitosan-pectin membranes is able to improve membrane hydrophilicity. It can also be seen from Figure 7 that membrane composition with 1.5% PVA addition (M3) is the highest percentage transport efficiency of creatinine.

The right blend of hydrophilic groups and hydrophobic membrane will result in a certain hydrophilicity values with optimum performance. Appropriate range values of hydrophilicity can make the membrane work in the transport process optimally. Hydrophilicity of the membrane plays a role in determining transport efficiency of a membrane. If membrane hydrophilicity achieves right composition so that the membrane will expand according to optimal water content capacity. Water contained in the membrane transport process is a means to permeate through the membrane. The optimum equivalent ratio of chitosan-pectin with PVA addition were determined by using the membranes for creatinine transport and water uptake percentage.

Conclusions

Chitosan/pectin/poly(vinyl alcohol) blend membranes were prepared successfully by inverse phase method. FTIR spectra at 1635.64 cm^{-1} of the membrane have indicated the interchain or intermolecular ionic salt bonds, i.e. polyelectrolyte complex (PEC) between amino groups of chitosan and carboxyl groups of pectin. Absorption at 1273.30 and 1327.07 cm^{-1} wave numbers only appears on the membrane spectrum, this is thought to be derived from an ester group showing cross-linking between alginate and PVA. Creatinin 70 mg/L which is transported by a membrane with 1.5% PVA addition yields optimum transport efficiency 59%.

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